A conserved function for Inp2 in peroxisome inheritance

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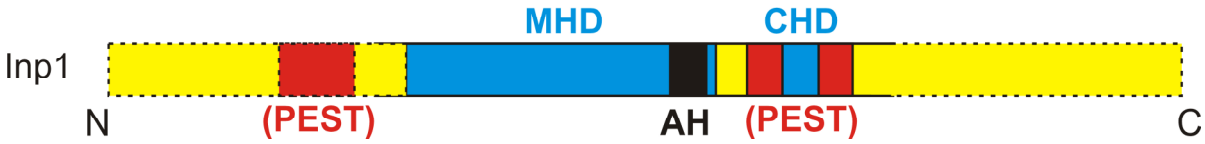
A.



B.

Middle Homology Domain (MHD):

```
Dh  : GLDMDKKVTLFKYKSSRILVFDEQLND---------------------- :  274
Ps  : NLNMDEKVTLFKYKSSKIIVVDEQHNE---------------------- :  293
Pg  : PLNINEKVTLFQFNSAKVVLYHEMVDS---------------------- :  229
Cl  : SYPMDDRTTIFRAPFARIVVYHEQVPD---------------------- :  129
Ca  : SLDLDEKVTLFKYKSSKILELVNQNN---------------------- :  198
Cd  : SLDLDEKVTLFKYKSSKILELVNQNN---------------------- :  193
Ct  : PQSLDDKINLFRYKSVEILEIANQNN---------------------- :  180
Le  : SSDIDAKVTLFKYKSLRIVELINQHT---------------------- :  269
Ag  : HSLSNDRVTLFRYEHVRVTSYQSV----RKKYRNSG-----DSRRGQERM :  129
Lt  : QRFSAQRVTLFKYEHVKVMNCNAS----SQRSNSDSSASTVTSQSTVLRR :  134
Vp  : KRSSAQRVSLFKYSNVQVVNCSAPLSPVQIRKDSVSSGSTSTSRNYDINS :  144
Cg  : KRLSAQRVALFKYNNVQVFNY--------VRNGNNSRKSSMSSLNSSGTV :  110
Sc  : KRISTQRFSLFTYGNVQVMNSFVPIHNDIPNSSCIRRNSQVSANNVTESS :  123
Zr  : QRLSAHKVSLFQYSNVKAISC------------------------------ :   51
Kl  : KRKSVQRTTLFRHDYVKVRSWPSDNPK---------------------- :   71
Hp  : FSHPSAKITLYEVSRINESATDQFQPG---------------------- :  134
Pp  : FEHPKCQMLLYEGNIDRRTPGNASARK---------------------- :  178
Yl  : TEPDEADPNLDPWEQLPPVHCAIS------------------------- :  124

Dh  : ----------NKTDMEKLSSGRLLGHGEFEIFQLHNG------------ :  301
Ps  : ----------SNS---NSSSGRLLGHGEFEVFQLHNG------------ :  317
Pg  : ----------NQS--IPKESGTLLGHGSFEVFQLHKG------------ :  254
Cl  : ----------SGIN---ASSGTLLGHGEFTIFQLHNG------------ :  153
Ca  : ------------------ANGSLLAHGIFEIFQLHQG------------ :  217
Cd  : ------------------VNGSLLAHGIFEIFQLHQG------------ :  212
Ct  : ------------------INGSLLAHGVFEVFQLHRG------------ :  199
Le  : ------------------QSGSLLAHGEIEIFQLHNG------------ :  288
Ag  : ESAVKGTD-----VLRRERASVTEPRGPLEIYQIITPISKEPS------- :  167
Lt  : SSSRDNPS-----SGSFKRETCLMSNGVLEIYQIITYNVKSPP------- :  172
Vp  : SLSVKSTS-----TVT-MKSISLVSHGVMEVYQILTPTKDPNE------- :  181
Cg  : VMCNEYDS-----EVTNLKPQMLIGKGVLELYQIKTP-AALDR------- :  147
Sc  : GVFFNDTQSQDSQNTIKLKPTSLMAKGPIEIYQICTGFDKLKENIAPFQK :  173
Zr  : -------------TDGNKNTSPLLSNGPLELYQIVTPAPNNYD------- :   81
Kl  : ------------------NINVILSKTSFEIYEIDMGNSK---------- :   93
Hp  : ------------------RNGNILGHGQFEIYQIQNK------------ :  153
Pp  : --------------------GKTVGQGKFKIYKMLKD------------ :  195
Yl  : --------------------ERIVAHGRFQVFTLHND------------ :  141
```

```
Dh  : ----------DVTYLSCG--PSFVYPLLPKLKILRINFN-----QFILPL :  334
Ps  : ----------DVTYLSCG--PSFIYPLFSKQKLLRIGFN-----QFILPL :  350
Pg  : ----------DVTYLSCG--SSFVYPLLPKLKILRTNFN-----QFILPL :  287
Cl  : ----------DITYLACG--PSFVYPLLRKLKILRVSRS-----HFILPL :  186
Ca  : ----------DVTYLSCG--NNFIYPLLPKIKVFRINSN-----QFLLPL :  250
Cd  : ----------DVTYLSCG--NNFIYPLLPKIKVFRINSN-----QFLLPL :  245
Ct  : ----------DVTFLSCG--STFVYPLLSKTKILRINSN-----QFMLPL :  232
Le  : ----------DVTYLSCGG-KLFIYPLLPKIKVLRISFN-----QFLLPL :  322
Ag  : ---------QKVTYLCLGRKEQIIKPILPKLRITMLTREG---LQFSVLS :  205
Lt  : ---------QKMTYLCLGRKGNIIHPILPRLQVTRLDSPE---FKISILL :  210
Vp  : -------TPQMMNYFSLGRNGKIVHPILPRLQVTRLHGYE---ASYSVLF :  221
Cg  : -------KEQTMNYISLGRGGQIVHPILPKLKITKLRNEN---FKYLITF :  187
Sc  : SSKASSHDGHVVNYLSIGRHGDIVHPVLPKLQITRLNGAG---FKYFISF :  220
Zr  : -------KSQEMNYLSLGRNGNIVHPILPRLQVKKLKTNG---VRFLITF :  121
Kl  : ---------ERMNYLSLGKKDQFVHPILPKLKVGRVPSDQKQEFKIISL :  134
Hp  : ----------SITYLSCG---SVVYPIMPRLKVLKISAS-----SFIIPL :  185
Pp  : ----------APYMKCG---HVVYPLLPKLRIIRIAMN-----QFIFPI :  226
Yl  : ----------KVTYIKCG---DAVQAILPKLRLWRTSLS-----QFIFPQ :  173

                                                            
Dh  : VNPERYWKISIN----SDDKNLIHLLERTLERNVKYRNLFFGINQLSSID :  380
Ps  : VNPERYWKIFIN----SDEPNVIEVLESSFERVVKYRNLYFKSKETETPR :  396
Pg  : ANPERYWRISLN----TEDVKIIRRLERTLEEKIQYRNFYLEDS------ :  327
Cl  : VNPQRYWKMEVV----SQEDAVLEELEKVFQKFVNYTSLFLVSSPVDDGE :  232
Ca  : VNPERYWKIFIN----SEELNVINNLINVFQRNVQFISLHESENKNA--- :  293
Cd  : VNPERYWKIFIN----SEELNVINNLINVFQRNVQFISLHESENKNT--- :  288
Ct  : TKPERYWKISLH----CDDFEVINSLVNVLQKNVQFISLYEQE------- :  271
Le  : LNPERYWKIYVD----CEDVSTLDCLEGVLKWNVQYINISNDKSDKSDKL :  368
Ag  : FNPENSWKIEFLGALGDSA--VPCNVILAFENAVKNICRYTSEL------ :  247
Lt  : FNPERFWEIEFLPSGELAK--LNDQIIFEFEGIISTICSYKSEK------ :  252
Vp  : YNPTRYWRIDFLPVENLVEKNQLNSILADFESVISSICEFSKESEYTIHN :  271
Cg  : SNPERYWQIEFLQINGQLH-DELLKITDEFESIISSVCIFIDEN------ :  230
Sc  : YNPERYWEIEFLPLISQSQ-SELENSVKAFENVISKICQFSHINEGATIG :  269
Zr  : YNPERYWELEFLPNNDDKD---LWAIVDDFEKIISKICVYKSVEEIR--- :  165
Kl  : FNPERFWEVTFLS---INHGRVPRKVINDLEQVLSKICQYNNKDPDN--- :  178
Hp  : SNPERYWKITLF----TEDAATLAQLDSVFKGICHFCAICDPPDAEELAE :  231
Pp  : HNPERYRQLVIN----SDDGEVLDKLELTFSQLCQYSTLFFPVP------ :  266
Yl  : PIPGRYWRVELFR----STYKIADDLRMALEQSCCFLDQIKAPELEEG-- :  217
```

C-terminal Homology Domain (CHD):

```
Dh  : FQNKAHKSQDNKSDSSMDSLLDEYEDNIHTTKS---ITFN-SRPPSRPIS :  591
Ps  : LAHSHQKSADHKSDSSMDSLLDEYEENINTTKS---ITFTRSRPPSRQHS :  596
Pg  : QYPSENGFHDRKSESSMDSLLDEFEENISITRS---ISFSAARPESHLPS :  509
Cl  : IANPYHHTSQQSDSSSMDSLLDEYEENVSMTRS---INYNGSRMPSRTAS :  403
Ca  : S--QKYPAIDEKSESSMDSLLDEYEENIHKS------MTIASRPQSRQPS :  473
Cd  : NYEKKYPAIDEKSESSMDSLLDEYEENIHKS------MTIGSRPQSRQPS :  474
Ct  : N---KYTTIDEKSESSMDSLLDEYEQNLNKS------TVINSRPPTRQPS :  424
Le  : SRHHHIDRIDEKSESSMDSLLDEFDDTVARTTKYPQTLSRQSRPVSRQQS :  368
Ag  : ------------DDDDLEYLLYS------------------------- :  269
Lt  : ------------NESDLEYLLEDSDESFDENVSITGSTCNKDDLIHDAFQ :  309
Vp  : TVKPIPSTEDVDDQDDLNYLLFEEEEQPQKDLKIYDDI-SRVNSISERIL :  414
Cg  : -----------DEAEELDYLLEDPIEQE--------------------- :  282
Sc  : --------------DDLNYLLDEEYEQGCTDN--------SFSVISNTCS :  337
Zr  : --------------DELDYLLNESEQAQDSNP----------------RL :  204
Kl  : ---------ILEEVDDLEYLLAENVPDQEQIPEAESLELNESSTISSISM :  238
Hp  : -----------HSMSSLNSTLACFNLSKDIETS--EADFLKHPTPIKLKS :  287
Pp  : -----------EEDVNLNVSGDIKDFNIIKISSSTPKKLERDLSISSDMA :  324
Yl  : TVEEVRTNGGELNMDFLASQIDNLSIPTHYDSEEHDLETIGSASSPYSSL :  319
```

Supplementary Figure 1

A. Schematic representation of Inp1-related proteins in budding yeasts.

Two homologous regions, designated the Middle Homology Domain (MHD) and the C-terminal Homology Domain (CHD), are indicated in *blue*. Two putative PEST regions implicated in protein degradation are indicated in *red*. Most Inp1 proteins have predicted PEST regions, but the loaction is not always conserved. A conserved region inside the MHD that has the ability to form an amphipatic helix (AH), is indicated in *black*.
Inp1-related proteins are highly diverse in size ranging from 287 to 944 amino acids. *Debaryomyces hansenii* and its relatives have large N- and C-terminal extensions (*dashed regions*). Only in these organisms the Inp1-related proteins mostly contain an N-terminal PEST region.

B. Alignment of amino acid sequences of yeast Inp1 proteins.

Protein sequences were aligned using Clustal_X and depicted by Genedoc. The one letter code is shown. Conserved residues are shaded. Only the conserved MHD and CHD regions of the proteins are shown. The line above the sequences indicates the amphipathic helix (AH) that is present in the MHD domain.
Ag, *Ashbya gossypii*; Ca, *Candida albicans*; Cd, *Candida dubliniensis*; Cg, *Candida glabrata*; Cl, *Clavispora lusitaniae*; Ct, *Candida tropicalis*; Dh, *Debaryomyces hansenii*; Hp, *Hansenula polymorpha*; Kl, *Kluyveromyces lactis*; Le, *Lodderomyces elongisporus*; Lt, *Lachancea thermotolerans*; Pg, *Pichia guilliermondii*; Pp, *Pichia pastoris*; Ps, *Pichia stipitis*; Sc, *Saccharomyces cerevisiae*;Vp, *Vanderwaltozyma polyspora*; Yl, *Yarrowia lipolytica*; Zr, *Zygosaccharomyces rouxii.* Sequence data were taken from the National Center for Biotechnological Information (NCBI), for accession numbers see **Table 2**.